

# A METHOD AND A DEVICE FOR DEFINING A SMALL VOLUME OF A LIQUID SAMPLE

The present invention concerns a method and a device for defining a small volume of a liquid sample by separating it from a relatively large undefined volume of said sample, and being useful, for instance, in blood testing, for providing an accurately defined volume of a blood sample.

In blood testing, it is of crucial importance to define an accurate volume of a blood sample, since such accurately defined volume is later on used for certain tests. The accurately defined volume of blood sample is normally diluted by an accurately defined volume of a diluent or a lysing agent, in order to obtain a dilution of typically 1:400 when white blood cells are concerned and typically 1:40000 when counting of red blood cells (RBC) is concerned, in the latter case the dilution normally taking place in two steps. It is obvious, that measurement of sample volumes and dilution liquid volumes must be performed in an accurate and repeatable way such that not only a correct degree of dilution can always be guaranteed but also a thorough and uniform mixing of the two volumes is ensured. Apparently, accurate measurement of sample volumes is the most critical step in the dilution procedure, since the volumes concerned are extremely small compared to the corresponding diluent volumes. A typical sample volume in state of art blood testing devices is 20  $\mu$ l.

State of art devices frequently utilize turning valves for defining a sample volume to be diluted. Examples of such devices are disclosed in WO 98/22797, WO 99/01742 and WO 03/44488. A turning valve comprises a cylindrical valve body rotatably received within a corresponding cylindrical aperture of a valve housing. A measuring channel having a defined

volume extends between opposed positions at the periphery of the valve body. By rotating the valve body between defined positions, the opposed ends of the measuring channel are put into communication with mouths of various channels in the cylindrical aperture of the valve body. Hereby, in one rotational position of the valve housing, the measuring channel may be filled with the defined volume of a sample flowing between two opposed first channel mouths at the inner periphery of the cylindrical aperture. Upon rotation to another rotational position, thereby separating the defined volume from the two opposed first channel mouths and simultaneously putting the opposed ends of the measuring channel in communication with two opposed second channels mouths at the inner periphery of the cylindrical aperture, flushing of the measuring channel with a diluting liquid in order to achieve a desired dilution ratio is enabled.

Although well-functioning, due to their relative complexity, rotating valves are not suited for the mass production desirable for economical production of disposable blood testing equipment.

The present invention aims at providing a simple and reliable method for separating at least one small defined volume of a liquid sample from a relatively large undefined volume of said sample as well as an equally simple, reliable and cheap device for carrying out the method, particularly suited for reproducible mass production.

The method according to the present invention involves the steps of providing in a surface of a first body at least one cavity having said small defined volume; applying said relatively large volume of said sample onto said surface and into said at least one cavity; relatively moving said first

body and a scraper means so that said scraper means passes said at least one cavity, thereby scraping a volume of said relatively large volume therefrom and leaving said small defined volume in said at least one cavity.

- 5 The device according to the present invention for performing the method includes a first body and a second body. In a surface thereof the first body has at least one cavity (depression, notch, indent, cavity, etching, blasting) having said defined volume. The second body includes an edge  
10 relatively slidable along said surface and over the cavity, thereby separating (scraping) an excessive volume of sample from said surface, leaving the defined volume within the cavity.

15 Embodiments of the present inventions will be described hereinafter, reference being made to the accompanying drawings, wherein:

- Figs. 1a and b are schematic sectional views showing the principle of a device according to the invention shown in a first and a second position, respectively;
- Fig. 2 is a perspective view schematically showing a first principal embodiment of the device;
- Fig. 3 is a similar view showing a second principal embodiment of the device;
- Fig. 4 is a similar view showing a third principal embodiment of the device;
- Figs. 5a and b are views similar to those according to Figs. 1a and b, but showing also a sealing element between the two bodies;

- Fig. 5c is an enlargement of a portion of Fig. 5b;
- Fig. 6 is a plan view showing an embodiment of a sealing element;
- Fig. 7a is a view similar to Fig. 1, but showing an alternative embodiment of the device having a separate body for receiving a sample;
- Fig. 7b is a plan view of the separate body of Fig. 7a;
- Figs. 8a, b and c are views similar to Figs. 1a and b, but showing three consecutive steps in the operation of a device having a separate scraper; and
- Fig. 9 a - e show various examples of cavity configurations and patterns.

The principle of a device embodying the present invention is shown particularly in Figs. 1a and b.

5 A first body 10 has a surface 11 in which is provided a cavity 12 having an accurately defined, small volume. An undefined, relatively large volume 13 of a sample, such as a blood sample, is applied onto the surface 11 such that it is ensured that the cavity is filled with sample.

10 A second body 14 has an edge 15 abutting the surface 11 of the first body. The bodies 10 and 14 are relatively slidable along the surface 11 as indicated by an arrow A, so that the edge, upon passing the cavity, scrapes or shears off a volume 16 of the sample along the surface 11 leaving just the accurately defined, small sample volume 17 within the cavity 12. This situation is shown in Fig. 1b.

In practice, the second body 14 has a surface 18 starting at the edge 15 and facing and abutting the surface 11 of the first body in a fluid tight manner. For the purpose of diluting the accurately defined, small sample volume 17, two channels 19, 20 extend through the second body and open in its surface 18 at positions enabling respective fluid communication with the cavity 12 as shown in Fig. 1b. The channels are shown here to extend in a V-shaped, converging manner towards the surface 18. Evidently, the channels may extend in other directions towards the surface 11 and the cavity 12 therein, including mutually parallel channel directions.

Conduits 21, 22, indicated by broken lines, connect a respective one of the channels 19, 20 with a respective one of receptacles 23, 24. The receptacle 23 is shown in Fig. 1a to contain a defined volume of a liquid 25, such as a diluent or a lysing agent. When the first and second bodies are in the relative position shown in Fig. 1b, flow from the receptacle 23 through the conduit 21 will be directed into the cavity 12, thereby flushing its volume 17 of sample and bringing it through the conduit 22 into the receptacle 24 to provide therein a volume of diluted sample having a defined dilution ratio. The volume of diluted sample may be brought to flow several times forth and back between the two receptacles to ensure proper mixing and dilution, but tests have shown that already one flushing provides a satisfactory result.

Depending on the kind of test to be performed and the dilution ratio desired, the cavity may typically have a volume of between 0,05 and 10  $\mu$ l, even if it is quite possible to provide cavity volumes of, e.g., between 0,02 and 20  $\mu$ l.

In practice, it may be useful to provide more than one cavity in the surface of the body 10, for instance one relatively small cavity (e.g. 0,05  $\mu$ l) and one relatively large cavity (e.g. 10  $\mu$ l), thereby enabling simultaneous dilution into two different dilution ratios. This is the preferred embodiment of the invention as regards blood analysis.

Examples of devices embodying this possibility are shown in Figs. 2 - 4.

The embodiment of Fig. 2 comprises a block-shaped housing 25 (second body) having therein a slot 26. A slide 27 (first body) has in an upper surface 28 a smaller cavity 29 and a larger cavity 30. The slot has an edge 31 closely abutting the surface 28. Two pairs of converging channels 32, 33 and 34, 35 extend through the housing 25 and open in a surface of the slot 26 at respective locations corresponding to the positions of the cavities 29, 30. Thus, a non-shown, undefined volume of a sample applied onto the surface 28 will be sheared off by the edge 31 upon sliding the slide 27 into the slot as indicated by an arrow B, leaving two accurately defined volumes of sample in the cavities 29 and 30 to be diluted as explained in connection with Figs. 1a and b.

In Fig. 3, the first body is a disc-shaped body 36 having in a surface 37 a smaller cavity 38 and a larger cavity 39. The second body is likewise a disc-shaped body 40 having at its circumference a cut-out portion providing access to the surface 37 of the first body and also providing at least one edge 41 abutting the surface 37. Two pairs of converging channels 42, 43 and 44, 45 extend through the body 40 and open in its surface abutting the body 37 at respective locations corresponding to the positions of the cavities 38, 39. Thus, a non-shown, undefined volume of a sample applied onto the

surface 37 will be sheared off by the edge 41 upon relatively rotating the two bodies as indicated by an arrow C, leaving two accurately defined volumes of sample in the cavities 38 and 39 to be diluted as explained in connection with Figs. 1a and 1b.

In Fig. 4, the first body is a cylindrical body 46 having in its peripheral surface 47 a smaller cavity 48 and a larger cavity 49. The second body is a block-shaped body 50 having one surface 51 concavely shaped in conformity with the cylindrical surface 47. An edge 52 of the surface 51 abuts the surface 47. Two pairs of converging channels 53, 54 and 55, 56 extend through the body 50 and open in its surface 51 abutting the body 47 at respective locations corresponding to the positions of the cavities 48, 49. Thus, a non-shown, undefined volume of a sample applied onto the surface 47 will be sheared off by the edge 52 upon relatively rotating the two bodies as indicated by an arrow D, leaving two accurately defined volumes of sample in the cavities 48 and 49 to be diluted as explained in connection with Figs. 1a and b.

In order to obtain adequate sealing, it is convenient to provide a sealing element 57 between the two bodies 10 and 14 as shown in Figs. 5a and b and more closely in Fig. 5c.

In this embodiment, an edge 57' of the sealing element takes the place of the edge 15 of the second body 14 in the principal embodiment of Figs. 1a and b, thus separating the sample volume within the cavity 12 from the remainder of the sample. The sealing element is received within a correspondingly shaped recess 58 in the body 14', thus providing a seal around the cavity and the mouths of the conduits 19, 20.

The sealing element has a through hole or aperture 59 providing communication between the two channels 19, 20 and the cavity 12 in the relative sliding position of the bodies 14' and 10 shown in Figs. 5b and c.

5 The second body 14' has a cut-out portion 60, thus guiding and entrapping the larger sample volume 13 upon relative movement between the two bodies (Fig. 5b). The cut-out portion terminates in a relatively narrow slit 61 (Fig. 5c) just before the active edge 57' of the sealing element 57.

10 As further seen in Fig. 5c, the portion 57'' of the sealing element 57 located between the aperture 59 and the edge 57' has a width  $w$  exceeding the width  $d$  of the cavity 12. Hereby, it is ensured that the mouth of the cavity is completely sealed as the portion 57'' of the sealing element passes over  
15 it upon relative movement of the two bodies.

As an alternative to the sealing element shown in Figs. 5a and b having side walls directed perpendicularly to the surface 11 of the first body 10, the aperture 59 is shown in Fig. 5c to have side walls 59' inclined approximately in conformity with  
20 the inclination of the channels 19 and 20.

Fig. 6 shows an embodiment of a sealing element 62 useful for sealing of two cavities, for instance the relatively small cavity 29 and the relatively large cavity 30 in the embodiment according to Fig. 2. For the purpose of illustration only, the  
25 locations of these cavities are shown in Fig. 2 with dashed lines. The sealing element has two apertures 63 and 64. In order to provide sealing of both apertures during the entire relative movement of the body 27 and the sealing element, the latter is substantially L-shaped, i.e., the apertures are  
30 aligned but the active edge of the sealing element is divided into two edge portions 62', 62'' such that the respective

portions of the sealing element located between the apertures and the edge portions have different widths  $W1$  and  $W2$ , respectively, both being greater than the respective cavity width  $d1$ ,  $d2$ , respectively.

5 I Fig. 7a, a separate body 65 is slidably movably placed between the first body 10 and the second body 14. It has in its surface 65a facing the second body 14 a sample receiving aperture 66 communicating through a channel 67 with a sample holding cavity 68 in its surface 65b facing the first body 10.  
10 Thus, at least a portion of a blood sample, for instance, applied into the aperture 66 will be transferred into the cavity 67. A through hole 69 extends through the body 65.

Upon relative movement of the body 65 and the first and second bodies, a portion of the sample volume contained within the  
15 sample holding cavity 68 will be entrapped within the cavity 12 in the first body 10, completely filling it. A trailing edge 68' of the cavity 68 will act as a scraping edge ensuring that superfluous amounts of sample is removed from the sample volume to be further treated. The through hole 69 provides for  
20 communication between the channels 19, 20 and the cavity 12. It should be noted here, that the relative dimensions between the various items shown and described are for illustrative purposes only.

In the embodiment shown in Figs. 8a - c, a separate scraper 70  
25 having a scraping edge 70' is provided and is used for scraping off superfluous sample from the surface 11 around the cavity 12. In this embodiment, the two bodies 10 and 14 are initially and during the scraping operation (Figs. 8a and b) spaced from each other. After the scraping operation is  
30 finished, the two bodies are caused to approach one another along an arcuate path as indicated by the arrow E in Fig. 8c

so as to bring the bodies in a position to perform the flushing operation.

Fig. 9 shows various cavity shapes and configurations. Fig. 9a shows a part-spherical shape, Fig. 9b shows a pattern of a plurality of substantially circular depressions, Fig. 9c shows a square or pyramidal depression, Fig. 9d shows a number of parallel grooves, and Fig. 9e shows a rectangular cavity shaped by, e.g., etching, blasting, cutting or the like material removing method.